United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract

Government Performance Results Act (GPRA) Goal 5
Annual Performance Measure 250

Significant Research Findings:

Microchip and Bio-Sensor for Detection of Phenols

Scientific Problem and Policy Issues

Research results described in this abstract contribute to the Safe Waste Management Goal (5) to reduce or control risks to health and the environment from Superfund and Resource Conservation and Recovery Act (RCRA) sites. As part of the effort to develop and characterize cost-effective technologies for remediation and characterization of contaminated soils, sediments, and ground water, this project has developed and characterized electrochemical sensors and biosensors for the screening of hazardous compounds such as phenol. Phenolic compounds have been reported at over 80 Superfund sites and are listed on the Agency for Toxic Substances and Disease Registry's (ATSDR) Priority Hazardous Substances List. Because these compounds are difficult to measure even under laboratory conditions, methods for screening these compounds in the field could be of considerable value. Research conducted for this project has focused on characterization of rapid and inexpensive techniques that are well suited for development as field analytical methods. Techniques demonstrated include microchip-based and biosensor-based methods for measurement of environmentally significant phenols (i.e., listed Superfund contaminants) using relevant matrices (i.e., ground water, soils, and sludges).

Research Approach

Since most phenols are oxidizable at moderate potentials, amperometry can serve as a highly sensitive tool for their detection and has been used to monitor phenolic compounds in conventional liquid-chromatography, flow-injection, and capillary electrophoresis (CE) systems. These analytical systems, however, are typically configured for laboratory methods which require large instrumentation and time-consuming protocols. The adaptation of micromachining technology to CE systems provides a significant potential for development of small field instruments. For this project, a chip-based CE system is demonstrated and characterized for the rapid separation and detection of 10 priority phenols. Another approach to rapid and inexpensive screening of phenols involves the use of the enzyme tyrosinase interfaced to an electrode to form a biosensor. This project characterizes a simple high performance liquid chromatography (HPLC) system that incorporates a biosensor detector for measurement of phenols. Due to its versatility with respect to incorporation of enzymes, a carbon paste electrode was used for the biosensor platform. The binder composition and viscosity of the carbon paste electrode was characterized and optimized for its response to

specific phenols. This system was demonstrated for 15 priority phenols as well as for phenol-contaminated leachate samples from two Superfund Sites.

Results and Impact

The microcapillary electrophoresis system used for this study was composed of a glass microscope slide with a micromachined 't' shaped channel (50 µm x 50 µm x 72 mm). This configuration, along with a small electrophoresis / detection instrument (5 in x 5 in x 8 in), was capable of separating and measuring phenol derivatives in about five minutes. These results were obtained using a laboratory prototype instrument, nevertheless, this report shows the feasibility of this technique for further development as a field methodology. The microcapillary electrophoresis system detected seven priority phenol derivatives with a linear range of 1-200 µM. A highly stable response was observed for repetitive analyses (RSD = 3.7, n = 20). The assay was also demonstrated for river water.

The HPLC system with the biosensor detector showed certain advantages and limitations compared with the chip-based CE system for development as a potential screening method. Although HPLC systems using amperometric detection are powerful analytical tools with respect to separation of closely related compounds, complex mixtures of organics typically yield a high number of oxidation peaks, each of which must be identified and calibrated. This complexity limits the potential development of this technique as a rapid field screening method. If the detector, however, were only sensitive to a limited number of compounds (e.g., certain phenols) which could be used as indicators for this class of compound, then this technique could be developed as a field screening method for these compounds. These studies characterize this technique with respect to extraction solvents, response to various phenols and electrode construction and composition. The HPLC system with a biosensor detector measures several indicator phenol compounds over the dynamic range of 2-30 ppm. The mechanism of the enzyme-facilitated electrochemical amplification was further confirmed by studies that controlled the viscosity of the carbon paste electrode binder; the use of silicon oil binders in the carbon paste increased the sensitivity by 6 times. This system is rapid, simple, and well suited for further development as a field assay.

This project directly supports Office of Research and Development (ORD) research to provide cost-effective technologies for characterization of soils, sediments and ground water under the Government Performance and Results Act (GPRA) Goal 5 (Safe Waste Management), Objective 1, Subobjective 6 (Conduct Contaminated Sites Research), Annual Performance Goal (APG) 9 (Provide at least 2 new soil sampling and on-site screening methods), Annual Performance Measure (APM) 250 (Microchip sensor [Biosensor] for detection of phenols – provide journal articles and reports to support field methods and biosensors for detection of phenols from contaminated Superfund sites).

Research Collaboration and Research Products

The phenol microsensor and biosensor projects were conducted by NERL scientists in collaboration with Dr. Joseph Wang at New Mexico State University (NMSU). This research has been presented at several National and International meetings as well as Regional and Program Office Seminars. Peer-reviewed

journal articles and U.S. Environmental Protection Agency reports resulting from this project are listed below.

Wang, J. Demonstration of the Feasibility of a Microchip-Based Device for the Separation and Detection of Phenols. Project Officer, K.R. Rogers, (EPA Report number pending), 2002. (in review)

Rogers, K.R., Cembrano, J. and Becker, J.Y. Viscosity and Binder Composition Effects on Tyrosinase-Based Carbon Paste Electrode for Detection of Phenol and Catechol. Talanta 54, 1059-1065, 2001. This study describes mechanistic implications of an enzyme electrode for phenols. A better understanding of this system will allow for the development and improvement of a field portable assay.

Rogers, K. R. & Becker J. Y. Improved Selective Electrocatalytic Oxidation of Phenols by Tyrosinase-Based Carbon Paste Electrode Biosensor. Electrochimica Acta 45, 4373-4379, 2000. This paper describes a simple and rapid assay using HPLC with a biosensor detector. The sensitivity of the carbon paste enzyme electrode was improved 6 fold by use of the silicon oil PS 086 as the electrode binder.

Rogers, K. R., Becker J. Y., Wang, J. & Lu, F. Determination of Phenols in Environmentally Relevant Matrices Using a Liquid Chromatographic System with an Enzyme-Based Biosensor. Field Anal. Chem. Technol. 3, 161-169, 1999. This paper describes a simple and rapid assay using HPLC with a tyrosinase-containing carbon paste electrode detector for the measurement of phenol, p-cresol, p-methoxyphenol, and p-chlorophenol in environmental matrices.

Rogers, K.R., Becker, J.Y., and Cembrano, J. Tyrosinase-based carbon paste electrode biosensor for detection of phenols: binder and pre-oxidation effects. 8th International Meeting on Chemical Sensors, Basel, Switzerland, July 2-5, 2000.

Future Research

Research results outlined in this abstract have characterized and demonstrated the feasibility of several screening methods for phenols. Based on these studies, several collaborations for further research are on-going. Projects include CE work with Dr. J. Wang at NMSU and phenol biosensor work with Smiths Industries (currently in the process of marketing a field portable electrochemical biosensor for phenols).

Contacts for Additional Information

Questions and inquiries can be directed to:

Kim R. Rogers, Ph.D.
U.S. EPA, Office of Research and Development
National Exposure Research Laboratory
944 E. Harmon
Las Vegas, NV 89119
Phone: 702/798-2299
Fax: 702/798-2107
E-mail: rogers.kim@epa.gov